In order to fully address the issues which were raised in the Office Action and discussed during the Interview, the Applicants submit herewith, pursuant to the provisions of 37 C.F.R. § 1.132, the Declaration of Dr. Michael Liskay. Dr. Liskay, a molecular biologist specializing in the field of DNA recombination and repair, is a Full Professor in the Department of Molecular Medical Genetics at the Oregon Health Sciences University in Portland, Oregon. Dr. Liskay actively conducts research in the field of molecular biology and genetics at the University, and one of his primary areas of research interests relates to the investigation of homologous recombination events in mammalian cells. Dr. Liskay has conducted research in this field for the past twelve years, and has published over twenty research papers on the topic of homologous recombination. (Liskay Decl. ¶ 1). In fact, Dr. Liskay is the first author of one of the references the Examiner has applied against the claims in this application. Dr. Liskay is eminently qualified to evaluate Dr. Skoultchi's invention, the applications filed by Dr. Skoultchi, and the art cited by the Examiner.

1. <u>The Invention</u>

The present invention involves the integration of a regulatory sequence and/or amplifiable gene into a mammalian host cell genome to activate and/or enhance expression of a target gene. A unique approach involving homologous recombination is utilized to engineer the target genes in mammalian host cells; however, the Applicants would like to stress that we are not attempting to patent homologous

recombination per se. In accordance with the invention, targeting vectors are designed and used to integrate a regulatory sequence different from the wild-type regulatory sequence and/or an amplifiable gene into a mammalian host cell genome in a region located within or proximal to a target gene of interest. Integration of the regulatory sequence in operative association with the target gene results in gene activation and/or enhanced expression; i.e., the integrated regulatory element, not the wild-type promoter, will drive expression of the target gene in the host cell. As aptly put by Mr. Charles Warren, the Group Director who attended the Interview, the wild-type promoter is "knocked out," and its function is replaced by the newly integrated exogenous promoter. Likewise, integration of an amplifiable gene proximal to the target gene allows for amplification of the target gene, which increases target gene expression.

The mammalian host cell so modified by targeted homologous recombination expresses the activated and/or amplified gene product. Where the engineered cell is a continuous cell line, it can be used for large-scale production of the gene product in cell culture. However, where the mammalian host cell is a primary cell that does not grow readily in culture, the activated gene can be transferred to a secondary expression host cell that is more efficient for large scale production.

As explained by Dr. Liskay, the invention affords a very unique, unusual and powerful approach to gene expression, obviating the need to clone the gene itself, and allowing for the expression of large genes that are difficult, or nearly

impossible to clone. Moreover, as pointed out by Dr. Liskay, the invention provides for the activation of normal human genes in normal, non-transformed human cells. Additional advantages are achieved, since unlike traditional recombinant DNA (rDNA) approaches to gene expression, e.g., where expression vectors or cassettes are used to direct the expression of cloned cDNAs, the invention provides for the expression of target genes which include all of their components, e.g., exons, introns, upstream and downstream sequences, that contribute to efficient expression of the gene product. (Liskay Decl. ¶ 7).

2. The Amended Claims

The present application is a Rule 60 continuation of application Serial No. 07/787,390 filed November 4, 1991 ("'390 parent application"), which, in turn, is a continuation-in-part of application Serial No. 432,069 filed November 6, 1989 ("'069 grandparent application").

The claims are fully supported by the '390 parent and '069 grandparent applications and have been amended to address the Examiner's rejections and concerns under 35 U.S.C. § 112. In particular, the term "regulatory element" is replaced with "regulatory sequence;" the term "modified" has been deleted; and the claims now specify that the regulatory sequences and/or amplifiable gene integrated within or proximal to the target gene does not disrupt transcription of the target gene. These amendments are fully supported by the specification as described infra in connection with the rejections under 35 U.S.C. 112.

Claims 28-31, 67-68, 71-72, 76, 83, 89-90, 93, 99 and 101 have been cancelled to eliminate redundancies.

The Applicants take this opportunity to respond to the Examiner's request expressed at the Interview to specify support for the pending claims which cover host cells (primary and secondary expression hosts) in which expression of the target gene is controlled and/or enhanced by the integrated regulatory sequence and/or amplifiable gene (Claims 26-47); methods for producing such host cells (Claims 48-66, 75, 77-82, and 84-88); and methods for producing recombinant protein using such host cells (Claims 69, 91, 92, 94-98, 100 and 102-104).

In particular, independent Claims 26, 48, 62, 69 and 97 and claims dependent therefrom relate to mammalian host cells (Claim 26), methods for producing such mammalian host cells (Claim 48), methods for producing secondary expression host cells (Claim 62) and methods for producing recombinant protein using such primary or secondary host cells (Claims 69 and 97) in which expression of the target gene is controlled by a regulatory sequence different from the wild-type regulatory sequence normally associated with the native target gene. The integration of regulatory sequences to control expression of the target gene is described in the '069 grandparent application (e.g., at p. 3, lines 16-21; p. 6, line 25 - p. 7, line 22) and the '390 parent application where it further is exemplified using the CMV promoter/enhancer to activate expression of EPO (e.g., at p. 3, lines 12-17; p. 7, lines 1-35; and examples at p. 15, line 1 - p. 19, line 20).

Independent Claims 32, 49, 63, 92 and 100 relate to mammalian host cells (Claim 32), methods for producing such mammalian host cells (Claim 49), methods for producing secondary expression host cells (Claim 63) and methods for producing recombinant protein using such host cells (Claims 92 and 100) in which expression of the target gene is enhanced by the integration of an amplifiable gene within or proximal to the target gene so that both the amplifiable gene and the target gene are amplified in response to selection pressure. The integration of amplifiable genes, such as DHFR, to amplify expression of target genes is described in the '069 grandparent application (e.g., at p. 3, lines 15-37; p. 4, line 36 to p. 5, line 10; p. 5, line 27 to p. 6, line 24; p. 9, lines 18-20; p. 10, lines 19-23; example, p. 12, line 1 to p. 14, line 17), and in the '390 parent application (e.g., at p. 3, lines 11-26; p. 4, lines 8-11; p. 5, lines 11-23; p. 6, lines 3-38; p. 9, lines 32-34; p. 10, line 34 to p. 11, line 2; examples at p. 12, line 20 to p. 19, line 20).

Dependent Claims 27, 75, 82, 91 and 98 cover cells and methods in which both an integrated regulatory sequence and an integrated amplifiable gene control or enhance expression of the target gene. The use of both regulatory sequences and amplifiable genes is described in the '069 grandparent application (e.g., see Summary of the Invention at p. 3, lines 15-29; p. 5, line 27 to p. 6, line 24) and is both described and exemplified in the '390 parent application (e.g., see Summary of the Invention at p. 3, lines 11-27; p. 6, lines 3-38; and example at p. 15, line 1 to p. 19, line 20).

The remaining dependent claims specify mutations that can be introduced into the target gene (Claims 33-36 and 58-61); mammalian host cells (Claims 37-42, 51-56 and 65-66); and selectable markers and negative selectable markers (Claims 50 and 79-81; and 64 and 86-88). These, likewise, are fully supported by the '069 grandparent and '390 parent application. Both applications describe a list of mammalian cells, primary and immortalized, that could be used in the invention ('069 grandparent application at p. 4, line 18-35; p. 10, line 31 - p. 11, line 4; and '390 parent application at p. 4, line 30 to p. 5, line 10; p. 11, line 10-20); mutations that could be introduced into the activated target gene ('069 grandparent application at p. 6, line 25 - p. 7, line 35; and '390 parent application at p. 7, lines 1-35); the use of selectable markers ('069 grandparent application at p. 9, lines 11-18; and '390 parent application at p. 9, lines 25-32); and the use of negative selectable markers ('069 grandparent application at p. 9, line 32 to p. 10, line 18; '390 parent application at p. 10, lines 10-31).

3. The Claims Are Entitled To The Benefit Of The November 6, 1989 Filing Date Of The '069 Grandparent Application Under 35 U.S.C. § 112 and § 120

The Examiner contends that only the claims covering enhancers as the transferred regulatory element are entitled to the priority benefits of the '069 grandparent application filing date because, according to the Examiner, "[t]here is no suggestion or teaching [in the '069 grandparent application] that heterologous regulatory elements other than enhancers

could also be transferred." The basis for this contention is erroneous, and all the pending claims are entitled to the benefit of the November 6, 1989 filing date of the '069 grandparent application.

At the outset, the '069 grandparent application specifically refers to "regulatory sequences" as sequences that can be targeted into a gene of interest via homologous recombination ('069 grandparent application at p. 3, lines 15-21). The '069 grandparent application then devotes at least two pages of the specification to a description of the regulatory sequences that can be targeted by homologous recombination in accordance with the invention. (See e.g., in particular, '069 grandparent application at p. 6, line 25 to p. 7, line 21). The foregoing written description found in the '069 grandparent application is identical to that of the '390 parent application (see '390 parent application at p. 3, lines 12-17 and p. 7, lines 1-35).

The written description in the '069 grandparent is clearly not confined to enhancers as contended by the Examiner, but includes the targeting of transcriptional initiation regions (i.e., promoters) different from the wild type transcription regulatory sequences of the target gene:

> "Alternatively, one could provide for insertion of a transcriptional initiation region different from the wild-type initiation region between the wild-type initiation region and the structural gene.
>
> * * *
>
> Therefore, by homologous recombination, one can provide for maintaining the integrity of the target gene, so as to express the wild-type protein under the transcriptional regulation of the wild-type promoter or one may provide for a

> change in transcriptional regulation, processing or sequence of the target gene. ('069 grandparent application at p. 6, line 28 to p. 7, line 14; emphasis supplied).

Surely, the Examiner must appreciate that targeting of promoters is intended by the description of the '069 grandparent application. In this regard, the Examiner's attention is invited to the Liskay Declaration. Dr. Liskay reviewed both the '390 parent application and the '069 grandparent application. (Liskay Decl. ¶ 3-6). Taking into account the knowledge and understanding of one skilled in the art of molecular biology would have had in 1989, Dr. Liskay concluded that the teachings of the '069 grandparent application are not limited to the targeted integration of enhancers, but include the targeted integration of any and all promoter elements different from the wild-type or native promoter that controls expression of the target gene. (Liskay Decl. ¶ 8).

First, the text itself refers to changing the transcriptional regulation region so that it is different from the wild-type promoter. Second, the specification's use of the term "transcriptional initiation region" is entirely consistent with the commonly accepted definition of a promoter. (Liskay Decl. ¶ 9). In this regard, the Examiner's attention is invited to the textbook definition of a promoter which was distributed and discussed at the Interview; i.e., a "[r]egion of a DNA molecule at which RNA polymerase binds and initiates transcription" (Old & Primrose, "Principles of Gene Manipulation, 2d ed., Univ. Calif. Press, Berkeley and Los Angeles 1981; emphasis supplied; Exhibit A attached hereto).

Indeed, the Applicants' definition is consistent with the descriptions and definitions of promoters supplied by the Examiner subsequent to the Interview attached hereto as Exhibit B (Watson et al., Recombinant DNA A Short Course, W.H. Freeman and Co., NY, 1983, pp. 41 and 47 (Exhibit B-1); Lewin, Genes, pp. 188-194 (Exhibit B-2); Liskay Decl. ¶¶ 10-11).

Based upon concerns expressed at the Interview, it appears that the Examiner is getting bogged down in the dissection of a promoter into its components; i.e., the RNA polymerase recognition site, the RNA polymerase binding site, and the transcription initiation site. However, the '069 grandparent application refers to the transcriptional initiation region, i.e., the promoter, as a whole. (Liskay Decl. ¶ 11). Indeed, the very title of the chapter in Lewin relied on by the Examiner, "Promoters: The Sites For Initiating Transcription," does not support the Examiner's strained interpretation.

In sum, the '069 grandparent application describes and identifies both promoters and enhancers as regulatory sequences that can be transferred into or proximal to the target gene, and does not stop there -- the '069 grandparent application additionally includes a description of the use of targeted homologous recombination to further modify the target gene of interest in order to improve expression and production of the gene product; i.e., the specification describes changing the signal leader sequence to promote secretion of the gene product, and changing the 3' region, e.g., the untranslated region, polyadenylation site, etc. of the target

gene ('069 grandparent application at p. 6, line 25 to p. 7, line 21).

In view of the foregoing disclosure in the '069 grandparent application as originally filed, it is clear that regulatory elements in addition to enhancers are adequately described and supported within the meaning of 35 U.S.C. § 112, and that all the pending claims are entitled to the priority benefit under 35 U.S.C. § 120 of the November 6, 1989 filing date of the '069 grandparent application.

4. The Claims Are Enabled And Definite Within The Meaning Of 35 U.S.C. § 112

Claims 26-44, 46-70 and 72-104 are rejected for non-enablement and indefiniteness under 35 U.S.C. § 112, first and second paragraphs. In particular, the Examiner objects to the terms "nucleotide regulatory element", as vague, unclear and not enabled by the specification, and "modified endogenous gene" as vague.

The Applicants believe that the amended claims obviate and/or overcome this rejection. First, the term "nucleotide regulatory element" has been replaced with "nucleotide regulatory sequence" -- a term which is specifically used in both the instant application and the '069 grandparent application, where it is defined as nucleotide sequences that control transcription of genes, e.g., promoters and enhancers (as discussed supra). The Examiner's objection to the term "modified" is obviated by deletion of that term from the amended claims. Thus, the rejection based on indefiniteness should be withdrawn.

At the Interview, the Examiner clarified her grounds of rejection for non-enablement. Specifically, the Examiner expressed concerns that the invention may not work in cases where the target gene is inaccessible for homologous recombination. However, as pointed out by the Applicants at the Interview, inaccessibility of the target gene in any given mammalian host cell does not present an obstacle to gene activation in accordance with the present invention. The present invention recognizes that when obstacles are encountered in one type of host cell, homologous recombination can be accomplished in a different host cell. (see Liskay Decl. ¶ 7). For instance, a primary host cell that expresses the target gene product, albeit in low quantities, can be used as the targeting host cell to ensure that the target DNA is in a conformation accessible for targeting. The activated target gene can then be transferred to a secondary expression host cell for large scale production of the gene product. For example, where a gene encoding a hormone is sought to be expressed at high levels, targeted homologous recombination can be carried out on a primary cell that ordinarily produces the hormone, albeit under strict regulatory control (e.g., in response to physiological signals) and in very small quantities. An additional advantage is realized when the invention is applied to human gene targets, because normal human genes can be activated in normal, untransformed human host cells. (Liskay Decl. ¶ 7). Once under the control of a strong constitutive or inducible promoter (i.e., integrated into the host cell genome via targeted homologous recombination), the recombined DNA, which contains the

activated target gene, can then be transferred to a secondary expression host cell line, e.g., a CHO (Chinese hamster ovary) cell line, for large scale production of the hormone gene product in cell cultures.

The versatility of the invention affords a powerful tool for gene activation that works. Moreover, the amended claims clearly exclude inoperative embodiments and are enabled within the meaning of 35 U.S.C. § 112, first paragraph. In particular, the amended claims specify that the regulatory sequence is targeted within or proximal to the target gene so that the structural gene or coding sequence is not disrupted and the target gene product is expressed. This amendment is fully supported by both the instant application and the '069 grandparent application, which specify that the regulatory sequence is introduced in proximity to the gene of interest, or within an intron of the target gene, without interruption of the production of a proper transcript (e.g., '069 grandparent application at p. 3, lines 16-21 and p. 6, line 28 to p. 7, line 21; and instant application at p. 3, lines 12-17 and p. 7, lines 1-35).

In view of the foregoing, all rejections under 35 U.S.C. § 112 should be withdrawn.

5. The Claimed Invention Is Not Obvious Under 35 U.S.C. § 103

The claims are rejected under 35 U.S.C. § 103 as obvious over Thomas either considered individually or in combination with Anderson, Song, or Liskay. The Examiner contends that Thomas discloses mammalian host cells having endogenous genes modified by homologous recombination so that

a regulatory element different from wild type is integrated into the genome, and thus renders the invention obvious. The Examiner further contends that Anderson teaches the use of enhancers in gene therapy and the use of homologous recombination; that Song provides the motivation to combine the references; and that Liskay differs from the claimed invention only in that it fails to disclose a secondary host cell. The Examiner contends that any deficiencies of Thomas are cured by Anderson, Song or Liskay and concludes that the claimed invention is obvious. For reasons detailed below, the Examiner's obviousness rejection is based on a misinterpretation of the teachings of the cited art and a flawed legal analysis of obviousness under 35 U.S.C. § 103. Therefore, the rejection is in error and should be withdrawn.

Subsequent to the Interview, the Examiner provided the Applicants with a copy of Thompson et al., 1989, Cell 56: 313-321 ("Thompson," attached hereto as Exhibit C and listed on the accompanying PTO Form 1449) indicating that she "intended to rely on this reference" as part of her analysis. However, Thompson is merely cumulative to the references of record and cannot support a rejection for obviousness, whether considered individually or in combination with the references of record.

5.1 <u>The Legal Standard For Obviousness</u>

A finding of obviousness requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the invention and the prior art, and whether the differences are such that the

invention as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere, 383 U.S. 1 (1966). Secondary considerations, which are indicia of nonobviousness, e.g., unexpected results, failure of others, etc. must be considered, if present. Fromson v. Advance Offset Plate, Inc., 755 F.2d 894, 904 (Fed. Cir. 1988). When references are combined to support an obviousness rejection, there must be some teaching from the references themselves that suggests the combination. In re Sernaker, 217 USPQ 1 (Fed. Cir. 1983). Once the scope and content of the prior art is determined, the relevant inquiry is whether the prior art both suggests the invention and would have provided one of ordinary skill in the art with a reasonable expectation that the invention would work. In re O'Farrell, 853 F.2d 894 (Fed. Cir. 1988). Both the suggestion of the change that led to the invention and the reasonable expectation of success must be found in the prior art and not in the Applicants' disclosure. In re Vaeck, 20 USPQ 2d 1438 (Fed. Cir. 1991). Finally, a prior art reference must be considered in its entirety; it is not permissible to disregard that portion of the prior art reference that diverges from and teaches away from the claimed invention. W.L. Gore & Associates v. Garlock, Inc., 220 USPQ 303, 311 (Fed. Cir. 1983).

Applying the foregoing legal principles to the present situation, the relevant question is whether the art relied on by the Examiner suggests that targeted homologous recombination should or could have been used to successfully activate gene expression in mammalian cells, e.g., by knocking

out the wild-type promoter/enhancer of a target gene and replacing its function with an exogenous, strong promoter/enhancer so that the exogenous promoter drives the expression of the endogenous gene. The simple answer to the question is no, and therefore, the invention is not obvious.

None of the art cited by the Examiner, whether considered individually or in combination, suggests the use of targeted homologous recombination to activate gene expression in mammalian cells for any purpose, much less large scale protein production in cell culture. Quite the opposite, Thomas, the primary reference relied upon by the Examiner, relates to the use of homologous recombination to "knock-out" gene expression by disrupting and inactivating the gene transcript. None of the secondary references relied on, including Thompson's vague hope of targeting unspecified "changes" to the control sequence of genes in embryonic stem cells, supplies the suggestion lacking in Thomas.

Moreover, there is no teaching or data presented in the cited art which would have provided one of ordinary skill in the art with a reasonable expectation of success, had the invention been suggested to him. Indeed, the art relied on by the Examiner teaches away from a reasonable expectation of success, in that it characterizes targeted homologous recombination in mammalian cells as a "formidable task" (e.g. see Anderson at p. 404).

Finally, Dr. Liskay, a molecular biologist who is eminently qualified in the field of homologous recombination, evaluated the foregoing references cited by the Examiner. (Liskay Decl. ¶¶ 12-17). Based upon his evaluation, and

taking into account the knowledge of one skilled in the art of molecular biology in the late 1980's, Dr. Liskay concluded that the cited art does not suggest the unique combination of elements as utilized in the invention (Liskay Decl. ¶¶ 18-24), nor would the cited art have provided one of ordinary skill in molecular biology in the late 1980's with a reasonable expectation of successfully activating gene expression for purposes such as large scale protein production in mammalian cell culture. (Liskay Decl. ¶¶ 25-29). Recognizing that Dr. Skoultchi's invention was a unique and unusual approach to gene expression for its time, Dr. Liskay concludes that Dr. Skoultchi's unique and unusual combination of elements used for targeted gene expression could only be said to be obvious in hindsight. (Liskay Decl. ¶ 30). The unobviousness of the claimed invention is perhaps best captured by Dr. Liskay's own words, "[t]he invention . . . affords a clever and powerful approach to gene expression that I wish I had thought of myself." (Liskay Decl. ¶ 7).

Thus, the rejection is in error and should be withdrawn. Each reference cited by the Examiner and the reasons for error are elaborated in the subsections below.

5.2. Thomas

Thomas describes site-directed inactivation of the endogenous Hprt gene in mouse ES (embryonic stem) cells by gene targeting. In particular, an rDNA construct prepared in vitro containing the bacterial neo^r gene controlled by the TK promoter was used to disrupt the endogenous Hprt gene by gene targeting using homologous recombination. The experiments

described by Thomas do not involve the insertion of a promoter (such as TK) to activate or control the expression of an endogenous gene of the host cell. Quite the opposite, Thomas describes the use of an exogenous gene (neo^r) controlled by an exogenous promoter (TK) to disrupt and inactivate the expression of the endogenous Hprt gene.

Referring to Thomas' use of the TK promoter and polyoma virus enhancer, the Examiner contends, "Thomas discloses a mammalian host cell having a modified endogenous gene after homologous recombination comprising a nucleotide regulatory element different from the wild type regulatory element and inserted into the genome of the mammalian host cell" (Office Action, p.6).

The Examiner's analysis is wrong. The pending claims specifically provide that expression of the target gene is activated or controlled by the integrated exogenous regulatory sequence which is in operative association with the target gene. This feature is not only absent from Thomas, but indeed, Thomas teaches the opposite. (Liskay Decl. ¶¶ 13 and 20). Hprt, the only endogenous target gene described by Thomas, is not controlled by a regulatory sequence different from the wild type Hprt promoter; the wild type Hprt promoter remains unaltered. Instead, Thomas attached the TK promoter to the exogenous bacterial neo^r gene via standard in vitro rDNA techniques. It is this rDNA construct that was used to disrupt and inactivate the endogenous Hprt gene in the mammalian cell. (The Examiner's attention is invited to the two handouts used during the Interview which diagrammatically

illustrate the differences between Thomas and the claimed invention, attached hereto as Exhibit D).

Thomas provides neither the suggestion of the invention, nor any support for a reasonable expectation of successful gene activation for purposes such as large-scale protein production in mammalian cell cultures. The Applicants' position is supported by the evaluation provided by Dr. Liskay. In particular, Dr. Liskay explains that Thomas does not suggest the use of targeted homologous recombination to integrate an exogenous regulatory sequence, such as a promoter, into the host cell genome to activate expression of the target gene; instead Thomas suggests the opposite -- i.e., disruption of a target gene so as to prevent expression. (Liskay Decl. ¶¶ 18-20).

Moreover, according to Dr. Liskay, the experiments reported by Thomas would not have led one of ordinary skill in the art at the time to have had a reasonable expectation of successfully activating gene expression via targeted homologous recombination in mammalian cells. (Liskay Decl. ¶¶ 25-27). As explained by Dr. Liskay, three types of homologous recombination events were known to occur in cells: extrachromosomal (e.g., plasmid/plasmid); intrachromosomal (e.g., chromosome/chromosome); and targeted (e.g., plasmid/chromosome). A cell type could be very efficient at performing one type of homologous recombination, (e.g., plasmid/plasmid) but relatively incompetent at targeted homologous recombination. (Liskay Decl. ¶ 2). At the time of the invention, targeted homologous recombination -- an event known to occur efficiently in bacteria and yeast -- was viewed

as a rare event in mammalian cells. (Liskay Decl. ¶¶ 25-26). However, the ES cells used by Thomas were viewed as an exception to the rule -- these embryonic stem cells were thought to possess unique capabilities to perform targeted homologous recombination, and in this regard were viewed as more akin to bacteria and yeast. Results achieved in ES cells were not extended or extrapolated to other mammalian cell types, least of which, human cells. (Liskay Decl. ¶ 27). Thus, based upon the results described in Thomas, i.e., the targeted inactivation of genes in ES cells, one of ordinary skill in the art at the time would not have had a reasonable expectation of successfully activating gene expression in mammalian cells via targeted homologous recombination. (Liskay Decl. ¶ 27).

In view of the Examiner's flawed analysis, all rejections under 35 U.S.C. § 103 based on Thomas should be withdrawn. The remaining references do not cure the defect. Thus, all obviousness rejections based upon combinations with Thomas should be withdrawn.

5.3 Anderson

Anderson is a review article entitled "Prospects for Human Gene Therapy." The article does not relate to gene activation, or gene expression for large scale protein production, much less how homologous recombination could be used for such purposes. Anderson does not even relate to modifying a resident gene by targeting. Instead, Anderson describes the progress and problems encountered when attempting to deliver rDNA constructs to cells in the body to

accomplish gene replacement therapy; i.e., the delivery of exogenous genes to cells in the body.

The Examiner makes much of the fact that Anderson mentions the use of enhancers in his rDNA constructs; however, this is irrelevant to the claimed invention. All the techniques described in Anderson involve classical in vitro rDNA technology to engineer the exogenous gene, and his focus is on the system used to deliver the exogenous gene to the host and/or somehow integrate the exogenous gene into the genome; e.g., using viral vectors, microinjection, and electroporation. (Liskay Decl. ¶ 17).

With respect to integrating exogenous genes into mammalian genomes for purposes of gene therapy, Anderson actually teaches away from the use of homologous recombination in mammalian cells; e.g., see Anderson, p. 404:

> The optimal system not only would deliver the vector specifically into the cell type of choice but would also direct the vector to a predetermined chromosomal site. Specific insertion into a selected site of a chromosome by means of homologous recombination can be readily achieved in lower organisms but appears to be a formidable task in mammals, whether retroviral vectors or plasmid-based vectors are used. Present evidence suggests that homologous site-specific integration occurs at a very low level, when it occurs at all, in mammals (citations omitted; emphasis supplied).

The foregoing was discussed during the Interview, where the Examiner expressed the opinion that "formidable" does not mean impossible, and concluded that Anderson supports a reasonable expectation of success, rendering the invention obvious. This analysis is erroneous for a number of reasons. At the outset, "not impossible" is not the legal test for obviousness. This was made abundantly clear at the Interview

by Mr. Richman, Director of the Patent Office. To support her obviousness rejection, the Examiner must point to something in the art that would have both suggested the invention and provided one of ordinary skill in the art with a reasonable expectation of success. In re Vaeck, supra. Anderson provides neither.

Moreover, the Examiner's interpretation of the foregoing quote from Anderson as supporting the use of homologous recombination in mammalian cells is completely at odds with the common ordinary meaning of "formidable:. . . 1. causing fear, dread or apprehension; 2. having qualities that discourage approach or attack; 3. tending to inspire awe or wonder" (Webster's New Collegiate Dictionary, G.&C. Merriam Co., Springfield, Massachusetts, 1991; emphasis supplied; attached hereto as Exhibit E). The Examiner's interpretation is also at odds with the evaluation and conclusions of Dr. Liskay -- a professor in the field of molecular biology with expertise in the study of homologous recombination, who is eminently qualified to evaluate the art; who did evaluate Anderson correctly; and who reached a conclusion opposite to that of the Examiner's. (Liskay Decl. ¶¶ 24 and 26).

As reflected by the above-quoted passage, Anderson clearly teaches away from the use of homologous recombination in mammalian cells, does not supply the suggestion proposed by the Examiner, and certainly does not provide any basis for a reasonable expectation of success. Quite the contrary, based on Anderson's teachings, one of ordinary skill in the art would have expected failure.

5.4 Song

Song describes homologous recombination events that occur between a defective neo^r gene, integrated into a host cell genome via random (i.e., not homologous) events, and a correct neo^r gene contained in a plasmid rDNA introduced into the cell via a secondary transfection. Song concludes that "[c]orrection of resident sequences by double crossovers, such as we and others have shown, could eventually be very useful for gene therapy." (Song, p. 6824 at col. 1, final paragraph).

The Examiner's contention that "Song provides the motivation to combine the references" because "Song discloses . . . that homologous recombination to correct resident sequences could eventually be very useful for gene therapy" (Office Action, p.8) misses the point. The claimed invention does not relate to the correction of mutant structural genes for gene therapy, but rather, to gene activation for any of a number of purposes, including large scale protein production in mammalian cell culture. Song does not relate to either gene activation or large scale protein production. Perhaps more importantly, Song does not suggest the use of homologous recombination to engineer exogenous promoters, enhancers, etc. into endogenous target genes of mammalian cells so that expression is controlled by the inserted element. (Liskay Decl. ¶¶ 15, 19 and 21).

Significantly, Song does not relate to the targeting of true endogenous genes, but rather to recombination events involving "resident" genes that are exogenous to the host; i.e., Song's "resident" genes are artificial targets

integrated into the host cell genome by random events. As explained by Dr. Liskay, success or failure of recombination between such artificial resident defective genes and their corrected counterparts borne on plasmids was not considered predictive of success or failure for recombination events involving endogenous native genes. Attempts to target mutations into endogenous native genes in mammalian cells revealed that the frequency of success was much lower than that achieved for Song's artificial targets, and was dependent upon the target gene and host cell -- e.g., whether the target gene was located within an inactive chromosomal locus (see, e.g., Smithies et al., 1985, Nature 317: 230-234, attached hereto as Exhibit F; and Liskay Decl. ¶ 28).

In sum, Song not only fails to suggest the invention, but since results obtained in Song's artificial system were not considered predictive of results for endogenous native genes that are used as targets in the invention, Song could not have provided a reasonable expectation of successfully using homologous recombination in mammalian cells to activate gene expression.

5.5 Liskay

Liskay describes a system for the study of intrachromosomal homologous recombination between duplicated genes in mammalian cells. Liskay's system involves the use of a plasmid containing two different mutations of a selectable gene, plus a separate dominant selectable marker that facilitates integration of the mutant pair into the genome -- this integration is a random event, not targeted, and does not

occur via homologous recombination. Thus, rDNA vectors constructed in vitro were used to prepare mammalian cells with duplicate genes which, in turn, were used to study recombination events between closely linked duplicated sequences lying in a chromosome in the mammalian cell. Liskay's purpose was to measure frequencies of both apparent non-reciprocal and reciprocal intrachromosomal recombination in cell lines containing a single direct repeat. (Liskay Decl. ¶ 16).

Liskay does not relate to targeted homologous recombination, much less its use for gene activation. This analysis is confirmed by Dr. Liskay himself. (Liskay Decl. ¶¶ 19, 23, 25, 26 and 29). As explained by Dr. Liskay, a cell which can perform intrachromosomal recombination may be incompetent to carry out gene targeting; thus, results achieved in one system cannot be extrapolated to another. Moreover, Liskay does not relate to the large scale production of proteins using mammalian expression hosts engineered by the insertion of regulatory sequences via targeted homologous recombination to activate or regulate the expression of an endogenous target gene.

In view of the foregoing, the claimed invention is not made obvious by Thomas whether considered alone or in combination with Anderson, Song, or Liskay. Therefore, the rejections under 35 U.S.C. § 103 should be withdrawn.

5.6 Thompson

Thompson was transmitted by the Examiner to the Applicants subsequent to the Interview, and has not been

applied against any of the claims. However, the Examiner has indicated that she intends to rely on Thompson. Thompson describes the use of homologous recombination to correct deletion mutations of the Hprt gene in ES cells. Cells from one corrected ES clone were introduced into mouse blastocysts, and germ line transmission of the ES cell-derived corrected gene was reported. The Examiner has highlighted the introduction of Thompson at column 1, which states:

> A strategy enabling precise modifications to be made to the mammalian genome would be of benefit both to biological and medical research. It would become possible to manipulate the expression of genes by targeting changes to their control sequences. This would be of value to the study of gene expression. It could also be potential commercial value, if used, for example, in livestock animals to increase output, or produce novel materials.

At the outset, it is abundantly clear that the work and data reflected in Thompson, i.e., correction of a deletion mutation in a structural gene in ES cells, does not suggest the invention. Moreover, the future hope reflected in Thompson's introduction, amounts to a mere wish to target unspecified "changes" to the control sequences of ES genes, and falls far short of suggesting the invention, much less providing a reasonable expectation of success. Simply put, Thompson does not suggest that an exogenous, strong regulatory sequence, e.g., a promoter, enhancer, etc. can or should be integrated into a mammalian host cell genome so that the wild-type promoter and/or enhancer of the target gene is "knocked-out," and expression of the target gene is instead controlled by the integrated promoter different from the wild-type promoter, i.e., gene activation, much less the use of such

cell systems for large scale protein production in mammalian cell culture. As explained by Dr. Liskay, when read in context with the entire article, it appears that Thompson's "changes" to the control sequences merely refer to targeting mutations to the Hprt promoter in order to "map" the promoter; i.e., to determine the mechanism by which expression of the Hprt gene is elevated in brain tissue (see Thompson, p. 319, col. 2, lines 55-58; and Liskay Decl. ¶ 22), W.L. Gore, supra.

Moreover, the data in Thompson would not have provided one of ordinary skill in the art a reasonable expectation of successfully manipulating gene sequences in mammalian host cells. In this regard, the Examiner's attention is invited to the Liskay Declaration at ¶¶ 25-27. As explained by Dr. Liskay, the prevailing view held at that time was that the ES cells used by Thompson (and others) were special, in that these embryonic cells possessed unique capabilities to perform targeted homologous recombination more efficiently than other mammalian cells. In this regard, the ES cell system, unlike any other mammalian cell, was viewed as more akin to bacterial and yeast systems which were believed to be much more efficient at targeted homologous recombination. Thus, any conclusions regarding feasibility of the system described by Thompson were confined to modifying ES cells in the hope of altering germ lines. Even Thompson did not project his results beyond mouse ES cells, when he admitted (Thompson, Summary, last sentence):

> We have demonstrated the feasibility of introducing targeted modifications into the mouse germ line by homologous recombination in ES cells.

And concluded (Thompson, p. 320, col. 1):

> Consequently, it should be possible to target modifications to any chosen gene in the mouse genomes.

In sum, when read in context with the entire reference, the isolated statement highlighted by the Examiner neither suggests the invention, nor does the reference provide a reasonable expectation of successfully activating gene expression in mammalian cell systems for large scale protein production as is taught by the invention.

The deficiency in Thompson is not supplied by the remaining references cited by the Examiner. As explained in the subsections above, none of Thomas, Anderson, Liskay or Song suggest the invention. (see Liskay Decl. ¶¶ 19-24). Moreover, assuming arguendo the invention had been suggested, none of the references would have provided a reasonable expectation of success. (see Liskay Decl. ¶¶ 25-30). Anderson clearly teaches away from the use of homologous recombination in mammalian cells, characterizing it as a "formidable task". Thomas, like Thompson, describes experiments involving homologous recombination in ES cells, the results of which could not be extrapolated to other mammalian cells. Song and Liskay describe experiments designed to study homologous recombination events involving artificial targets; i.e., exogenous target genes randomly integrated into the host cells genome. Moreover, Liskay's system related to intrachromosomal recombination, not targeting homologous recombination. Results obtained in these artificial systems, likewise, were not predictive of targeted homologous recombination events involving native, endogenous

genes. Indeed, homologous recombination events involving native, endogenous genes were known to be very rare -- any successes achieved were modest and dependent upon the target gene and host cell. Results were truly unpredictable.

5.7 Summary of Nonobviousness

In sum, none of the references cited by the Examiner suggest the invention; i.e., the regulation of expression of an endogenous target gene in mammalian host cells controlled by an exogenous promoter/enhancer, e.g., using targeted homologous recombination to "knock out" the wild-type endogenous promoter and replace its function with an integrated exogenous promoter, different from the wild-type promoter. None of Thompson, Thomas, Anderson, Song or Liskay provide the suggestion of the invention. Indeed, Thomas, the primary reference relied on by the Examiner, describes the opposite; i.e., the use of homologous recombination to knock out a structural gene and thereby prevent its expression. Thompson's vague hope of targeting unspecified "changes" or mutations to control sequences of target genes in ES cells is a far cry from suggesting knocking out the wild-type promoter, and replacing its function with an exogenous promoter, different from the wild-type, to drive expression of endogenous target genes in mammalian cells. Anderson relates to gene therapy using standard rDNA approaches to insert exogenous genes into host cells. Song and Liskay describe studies of homologous recombination events involving artificial targets, i.e., exogenous target genes inserted into

the host cell genome via random events. Liskay does not even involve targeted homologous recombination.

Assuming arguendo the invention had been suggested to one of ordinary skill in the art, none of the cited references would have provided a reasonable expectation of successfully activating target gene expression in mammalian cells. Targeted homologous recombination, which was known to occur efficiently in lower organisms such as bacteria and yeast, was viewed as a rare event and difficult task in mammalian cells. Thus, there would have been no motivation to use targeted homologous recombination to engineer target genes as taught by the Applicants. By analogy, the knowledge that lightning strikes and generates electricity would hardly provide the motivation to harness the energy generated by this rare event to supply the power to a city.

That homologous recombination was viewed as a rare event in mammalian cells is confirmed by Anderson who teaches away from the use of homologous recombination in mammalian cells, characterizing it as a "formidable task." Dr. Liskay has put into context the results reported in the remaining references in view of the understanding of one of ordinary skill in the art in the late 1980's. As explained by Dr. Liskay, both Thomas and Thompson reported studies involving homologous recombination in mouse ES cells -- a cell type viewed at the time of the invention as an exception to the rule. Results obtained in the mouse ES system were not extended, extrapolated or considered predictive of results that could be achieved in other mammalian cells. Song and Liskay describe studies of homologous recombination events

involving artificial targets; i.e., exogenous genes integrated into the host cell genome via random events. Results described in Song and Liskay's artificial system were not extrapolated or considered predictive of the frequency and success of homologous recombination events involving native target genes endogenous to the host cells genome. Moreover, Liskay involved the study of intrachromosomal, not targeted, homologous recombination events. Liskay's results, therefore, were not extrapolated to targeted homologous recombination, because it was understood that a cell could be capable of performing intrachromosomal or extrachromosomal recombination, yet incapable of carrying out targeted recombination.

In view of the foregoing, the art relied on by the Examiner does not render the claimed invention obvious.

5.8 The Examiner Has Engaged In Hindsight Reconstruction

The Applicants believe that the Examiner has, perhaps unwittingly, engaged in hindsight reconstruction, using the claims as a "blueprint" to pick and choose from the art in an attempt to recreate the invention -- a practice forbidden in a proper analysis under 35 U.S.C. § 103. In re Fine, 837 F.2d 1071 (Fed. Cir. 1988).

That hindsight is being used is supported by Dr. Liskay's analysis. Recognizing that each of the tools utilized in the invention was known individually, Dr. Liskay also appreciates and recognizes that the unique combination of elements used in Dr. Skoultchi's invention was not suggested by the art. Thus, concludes Dr. Liskay, the invention could only be said to be obvious with hindsight, after having

learned of Dr. Skoultchi's clever, unique and powerful approach. (Liskay Decl. ¶¶ 7 and 30).

The inappropriateness of hindsight as a test of obviousness was articulated by Milton over 300 years ago, in Paradise Lost, Part IV, L. 478-501, and was recently iterated by the Federal Circuit in Gillette Co. v. S.C. Johnson & Son, Inc., 919 F.2d 720, 726 (Fed. Cir. 1990):

> The invention all admired, and each how he
> To be the inventor missed; so easy it seemed,
> Once found, which yet unfound most would have thought,
> Impossible!

Heeding the Federal Circuit's admonition against the use of hindsight, it appears that Dr. Liskay's reaction after learning of the invention -- a response reflecting perhaps the highest accolade one investigator can pay to another -- evidences the true non-obviousness of the invention, "...I wish I had thought of [Dr. Skoultchi's invention] myself." (Liskay Decl. ¶ 7).

For all the foregoing reasons the claimed invention is not obvious, and the Examiner's rejections under 35 U.S.C. § 103 should be withdrawn.

6. Supplemental Information Disclosure Statement

The Applicants submit herewith a Supplemental Information Disclosure Statement and PTO Form 1449 listing all references cited in connection with the prosecution of the grandparent and parent applications, the foreign counterpart applications, the Chappel patent (United States Patent No. 5,272,071) and other references cited therein which are not already of record in the file of the instant application.

Each reference is listed on the accompanying PTO Form 1449, and copies are provided.

7. Conclusion

Entry and consideration of the foregoing amendments and remarks into the file of the above-identified application is respectfully requested. The Applicants believe that all claims are allowable, and request withdrawal of all rejections. Applicants renew their Request for Interference with United States Patent No. 5,272,071 granted to Chappel.

Respectfully submitted,

Dated: Feb 6, 1995

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